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VARIATION OF FUNGI DUE TO ENVIRONMENT[1]

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(WITH THIRTY-SEVEN FIGURES)

The effects of environment, climatic condition, soil fertility, the presence of unusual chemicals, the water relation, and what not, upon the form and characters of seed plants, are well known to the plant physiologist, and have been the subject of numerous studies. These factors are even utilized by the practical man to bring about desired variation.

That fungi vary similarly will not be doubted by any who have had to do with fungi in artificial cultures. The kind and degree of such variation, we dare say, will be a surprise to any who have not made special study of this subject.

Our knowledge of the seed plants, owing to man's long acquaintance with them, their larger size, and comparative stability, is considerable; yet even with them the limiting of genera, species, varieties, etc., presents difficulty, if we may judge from the rich literature upon phanerogamic taxonomy. The fungi, because of their immense number of species, variety of forms, minuteness, paucity of distinguishing characters, complexity of life-history (mostly unknown), peculiar biologic host relations (almost entirely unknown), and because of man's short acquaintance with them and their unknown but apparently vast range of variability, present as yet baffling problems of relationship and classification.

The object of the present paper is to call attention to the kind and degree of environmental variation found in a few species of fungi that have been studied by the authors during the past four or five years,

[1] Read in part at the Baltimore meeting of the Botanical Society of America, December, 1908.

and in some instances to analyze the causes of these variations, to the end that the factor of environmental variation may be more clearly recognized as a problem of mycological taxonomy.

We shall consider these variations under the causes that produce them.

I. Density of colonies

SEPTORIA PETROSELINI DESM. VAR. APII BR. & CAV., FROM CELERY

This fungus, when plated so that the spores lay thinly scattered, produced colonies which were ultimately black, 1 to 2^{mm} in diameter,

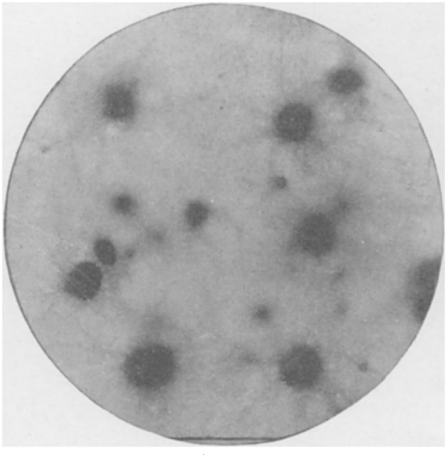

FIG. 1

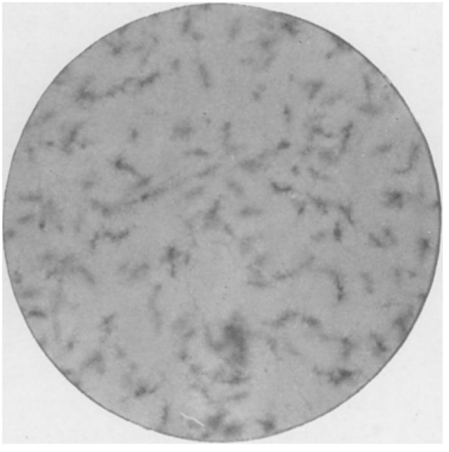

FIG. 2

FIG. 1. *Septoria Lycopersici* Speg., showing formation of normal pycnidia on portion of thinly sown plate culture.—FIG. 2. *Septoria Lycopersici* Speg., showing absence of pycnidia on thinly sown portion of plate culture; magnification same as in *fig. 1*.

with pycnidia of normal character. If plated so that the spores lay in large numbers per square centimeter, it produced colonies which reached a size of only about 0.5^{mm} and became ultimately black, containing ordinary pycnidia, bearing spores in the normal way. When plated so that there were still more spores per square centimeter, the colonies never became black and no pycnidia were produced; but on the contrary, multitudes of spores were borne uncovered, in clumps upon simple hyphae.

SEPTORIA LYCOPERSICI SPEG., FROM TOMATO

Spores from pure culture were plated in 4 per cent. pea agar in various dilutions.

One plate developed 5 to 6 colonies per square millimeter and each colony proceeded to normal pycnidial development. Another plate developed 21 to 23 colonies per square millimeter, and all proceeded to form naked conidia with no indication of pycnidia. Portions of these two plates are represented by photomicrographs (*figs. 1, 2*). Drawings of the naked spores showing the detail of their formation are given in *fig. 3*. Occasionally plates with as many as 30 colonies per square millimeter were found with both pycnidia and naked spores.

Pycnidia not visible at the fifth day may be well formed by the eighth day and extrude masses of pink spores about the twenty-first day. Occasionally pycnidia are well developed on the fourth day. When naked spores develop they normally appear a few days later than do pycnidia, e. g., a plate thinly sown January 12, 1907, gave many pycnidia on January 15; while a thickly sown plate, under conditions otherwise precisely parallel, did not give naked spores until January 22. This Septoria forms a typical determinate colony, i. e., even with unlimited room, it proceeds only to a certain size of development.

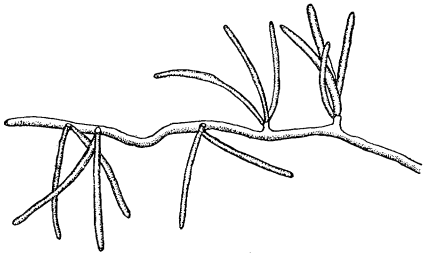

FIG. 3.—Mode of formation of naked spores under influence of crowded culture.

SEPTORIA CONSIMILIS E. & M., FROM LETTUCE

When sown thinly, colonies reached a size of 2 to 3^{mm} in diameter; when sown thickly, they became no more than 0.2^{mm} in diameter. There was no interference with color development or formation of pycnidia by thick sowing with this species.

With two of these septorias, thick plating, other conditions being the same, so changed their character that not only would the species be considered as different, but the fungus would be shifted from the order Sphaeropsidales to the order Hyphomycetales (Hyplaesa of SACCARDO).

A similar change of habit is well known in the genus Fusarium, which in culture, crowded or not, often abandons acervulus formation, thus changing its systematic position from the Tuberculariaceae to the Mucedinaceae. The genera Colletotrichum and Gloeosporium similarly abandon acervulus formation and thus suffer still greater taxonomic disturbance by moving from the Melanconiales to the Hyphomycetales.

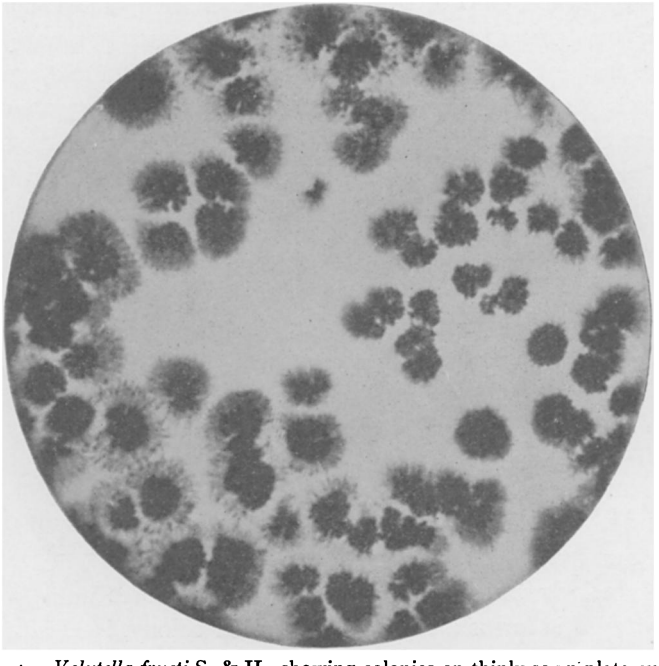

FIG. 4.—*Volutella fructi* S. & H., showing colonies on thinly sown plate culture.

ASCOCHYTA CHRYSANTHEMI STEVENS, FROM CHRYSANTHEMUM

This fungus was plated January 12, 1907. Myriads of pycnidia were present four days later. Thick plating caused no inhibition of pycnidial formation, no naked spores, and no constant effect upon the number of pycnidia produced.

VOLUTELLA FRUCTI S. & H., FROM APPLE

Thinly sown, the colonies were large, of indeterminate growth, showing dark centers with pale borders (*fig. 4*). Thickly sown, growth was inhibited and these characters lost (*fig. 5*).

SPERMOEDIA PASPALI FRIES, FROM PASPALUM[2]

Spores of this fungus were sown January 19, 1907, in plates giving colony densities of 90, 54, 30, 14, and 1 per square millimeter.

At all of these densities germination was practically 100 per cent. and growth proceeded equally in all plates during the early stages. On February 11 it was noted that all colonies which came nearly in

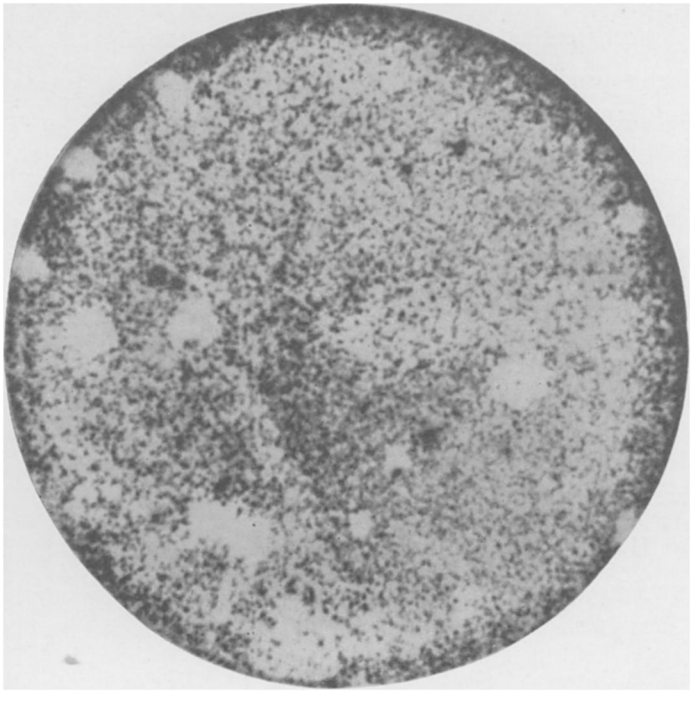

FIG. 5.—*Volutella fructi* S. & H., showing effect of thick sowing.

contact were sporing. Growth then stopped. In the plates bearing only one spore per square millimeter the colonies continued to enlarge slowly and to produce many spores in the central portion, though remaining white, not attaining the usual yellow color. Deep colonies appeared like the superficial, but bore no spores. On February 7 the colonies on thin plates (1 per square millimeter) had attained a

[2] Later study has shown this to be the imperfect stage of an undescribed species of Claviceps, which we shall describe in a subsequent paper.

diameter of 1.5^{mm}. Some of these colonies transferred to tubes continued to enlarge, became tubercular, and developed a yellow center 3 or 4^{mm} in diameter. The whole colony often reached 1^{cm} in diameter. Sister colonies left in the plate (1 per square millimeter) failed so to develop, and it is evident that at even this density normal development is not attained.

The colony is indeterminate in growth, and in plates its size is limited by the presence of adjacent colonies.

SUMMARY REGARDING THE DENSITY FACTOR

This factor produces different effects with the different species. It may inhibit pycnidial formation, resulting in naked spores; it may cause failure to develop color; it may limit the size of the colony; it may be without effect.

There are many paired species of the imperfect fungi agreeing closely, except in the presence or absence of one character. These pairs often occur upon the same host, e. g., *Septoria Lycopersici* Speg. and a Cylindrosporium on the tomato; *Cylindrosporium Chrysanthemi* E. & D. and *Septoria Chrysanthemi* Cav. on the cultivated chrysanthemum.[3] Many other instances could be cited. The lack of fixity of such a structure as even the pycnidium throws doubt upon the validity of such species as these and indicates the necessity of close comparative study.

II. Density of mycelium: zone formation

The formation of concentric zones is by many fungi one of the most conspicuous characters shown in culture. These zones may be

3 VOGLINO, P., Diseases of cultivated chrysanthemums. Malpighia **15**:329–341. 1902. (Exp. Sta. Rec. **14**:777.)

HALSTED, B. D., Chrysanthemum leaf spot. Amer. Florist **10**:263. 1894. (Exp. Sta. Rec. **6**:311.)

BEACH, S. A., Leaf spot of chrysanthemum. Report of Horticulturist, N. Y. Exp. Sta. Rept. **1892**:557–560.

HALSTED, B. D., Report of fungus disease of plants. N. J. Exp. Sta. Report **1891**:233–340.

SACCARDO, Syll. Fung. **2**:542, *n.* 3497, 3498, 3757.

TUBEUF & SMITH, Diseases of plants 478.

Year Book U. S. Dept. Agr. **1906**:507.

New York Agr. Expt. Sta. Rept. **14**:529.

New Jersey Agr. Expt. Sta. **1894**:361.

due to any one of many structural characters of the colony; to varying density of spore massing, grouping of pycnidia, mycelial branching, color, etc. It is a frequent phenomenon in nature in the fairy rings of the toadstools, the concentric markings of many leaf spots, fruit rots, etc. These effects have been attributed to various causal agencies, to light relation,[4] to nutrients,[5] to agencies other than light, probably food, to resting periods (HEDGECOCK, *l. c.*), and to mycelial crowding.[6]

ASCOCHYTA CHRYSANTHEMI STEVENS

With the fungus in question the fact that the zones are not due to light or temperature relations is apparent from the fact that they do not coincide with the fluctuations of these two factors (*fig. 6*). In the colony shown, which is that of a plate culture kept at room temperature, there was daily change from warm to cool, light to dark; yet the number of rings does not coincide with the number of these changes; moreover, zones were produced in precisely the same way on plates kept constantly in the dark as on plates kept all of the time in the light, and still the same on plates kept three days in the dark and then three days in the light.

Microscopic examination shows that with this fungus the dark zone is due to a larger number of mycelial filaments, the light zone to a smaller number of threads, as is shown diagrammatically in *fig. 7*. It seems that with this fungus the dense crowding of the filaments, resulting from their repeated branching, inhibits growth either by the products of metabolism or exhaustion of nutriment. There is then a period of quiescence, followed by onward growth of a few scattered hyphae. As these outgrowing hyphae reach beyond the inhibiting influence, they branch repeatedly, until a new dense zone

[4] MOLZ, EMIL, Ueber die Bedingungen der Entstehung der durch *Sclerotinia fructigena* erzeugten Schwarzfaule der Aepfel. Cent. Bakt. **17**²:175.

HUTCHINSON, H. B., Ueber Form und Bau der Kolonieen niederer Pilze. Cent. Bakt. **17**²:602.

HEDGECOCK, G. G., Zonation in artificial culture of Cephalothecium and other fungi. Rept. Mo. Bot. Garden **17**:115–117. *pls. 13–16.* 1906.

[5] MILBURN, THOS., Ueber Aenderungen der Farben bei Pilzen und Bakterien. Cent. Bakt. **13**²:257.

[6] ISTVÁNFFI, GY. DE, Études microbiologiques et mycologiques sur le rot gris de la vigne. Ann. Institut Cent. Ampél. Roy. Hongrois **1905**:183.

is formed. This process is repeated indefinitely. The rapidity of succession of zones is dependent solely upon the relation which rapidity of branching bears to rapidity of increase in length. Slow lineal

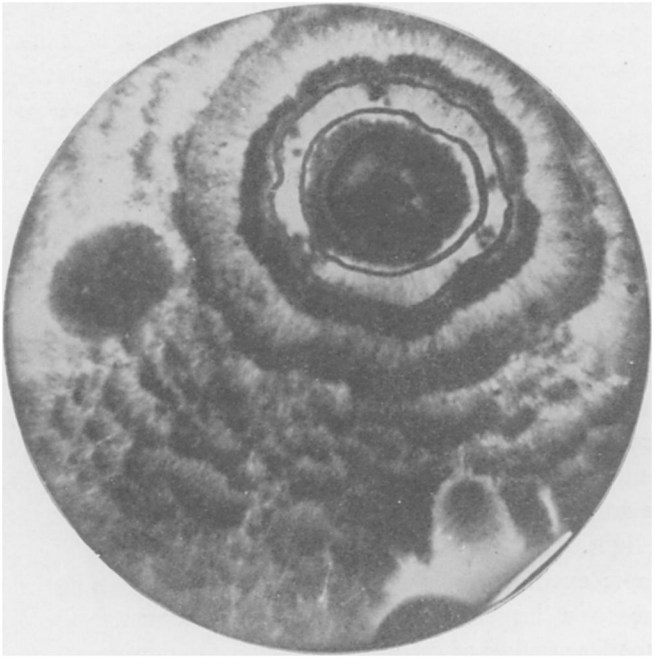

FIG. 6.—*Ascochyta Chrysanthemi* Stevens; plate culture showing that the formation of zones is not coincident with diurnal changes; ink marks show growth for three consecutive days.

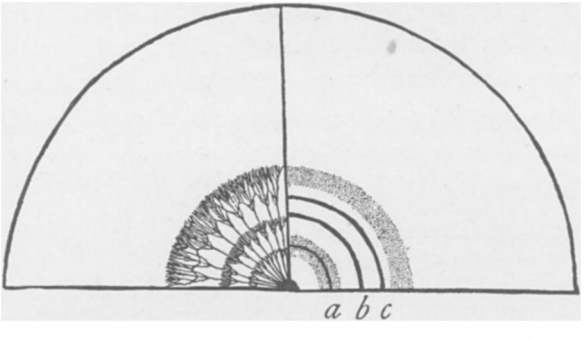

FIG. 7.—Diagram showing, at right, the zones (stippled) and diurnal marks; at left, theoretical expression of cause of zonation.

growth and much branching gives many narrow zones, rapid lineal growth with infrequent branching causes few broad zones.

Sclerotinia Libertiana Fuckel, from lettuce

Zonal sclerotial formation is exhibited by this fungus (*fig. 8*). That this phenomenon may be attributed to crowding of the mycelium is indicated by the fact that adjacent colonies form more sclerotia at their points of contact (*fig. 9*).

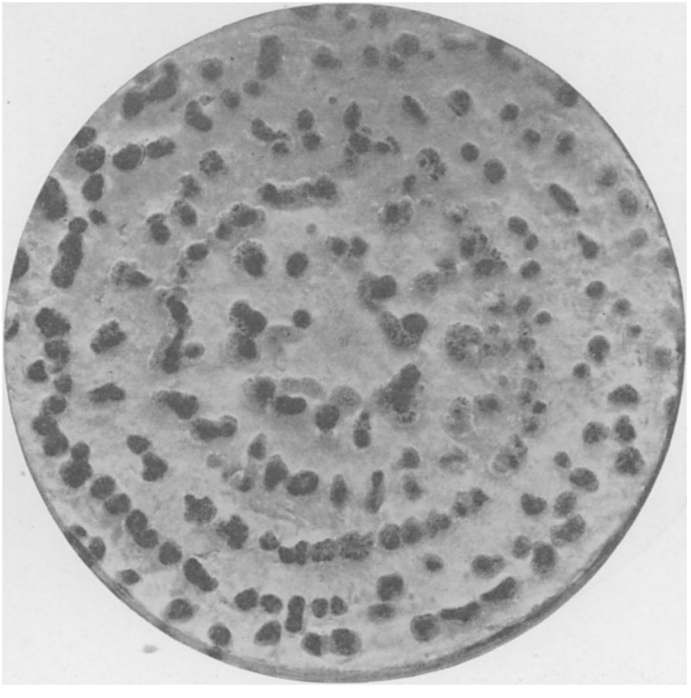

Fig. 8.—*Sclerotinia Libertiana* Fuck., showing zonal formation of sclerotia on corn-meal culture.

SUMMARY REGARDING DENSITY OF MYCELIUM

Zone formation in *Ascochyta Chrysanthemi* is due to crowding of mycelium, not to light or heat relation. A similar conclusion was reached by Istvánffi[6] regarding the very striking zones shown by Sclerotinia. The same cause may apply also with *Daldinia concentrica* and many other fungi of similar structure.

III. Chemical relations

Chemical relations have been studied with eleven fungi, the fungus being usually grown in agar with varying nutrients added. Occasionally other media were used. A chemical base agar (CBA) was

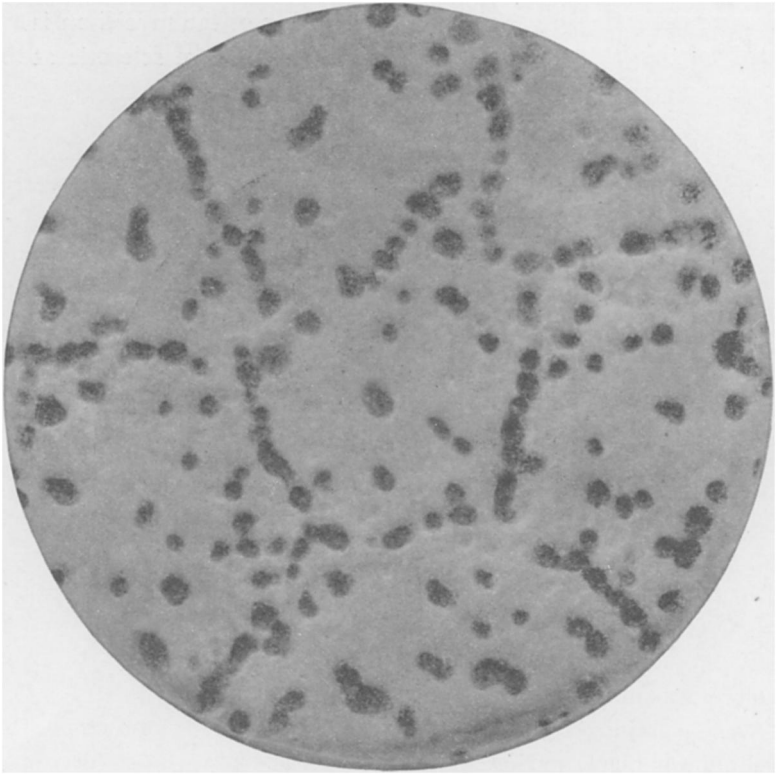

FIG. 9.—*Sclerotinia Libertiana* Fuck., showing the formation of sclerotia in greater abundance where adjacent colonies come in contact.

made of the following proportion (grams); water 1000, di-potassium phosphate 2.5, magnesium sulfate .01, calcium chlorid .01, sodium chlorid 2.5, potassium sulfate 2, agar 15. To 100^{cc} of this chemical base agar were added the following materials (grams) singly or in varying combinations: ammonium lactate 0.5, sodium asparaginate 0.25, glucose 1, starch 1. The tests were usually made in both plate and tube cultures.

VOLUTELLA FRUCTI S. & H., FROM APPLE

This fungus, when sown thin, forms large indeterminate colonies, often with numerous scattered tubercular blotches (*fig. 10*).

On pure agar and CBA the colonies were pale, mycelium hyaline, black tubercles very sparse.

On pea agar, black tubercles were much more abundant, otherwise as on pure agar.

On CBA+sodium asparaginate, black tubercles were still more numerous.

On CBA+sodium asparaginate + starch, black tubercles were more numerous than in any of the above, and the colony was black (*fig. 11*).

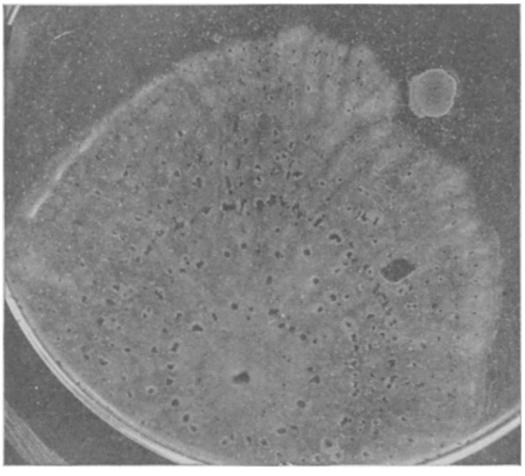

FIG. 10.—*Volutella fructi* S. & H.; colony on pea agar showing tubercular blotches, some of them in concentric rings; mycelium nearly hyaline, due to lack of carbohydrates.

On CBA+sodium asparaginate+glucose, black tubercles were still more numerous, so many as to be contiguous, and the whole colony was densely black.

On gelatinized starch, and starch+Uschinsky's solution, the mycelium was black and some digestion of the starch was observed.

On none of the above media were spores formed, but on sterilized apple twigs spores were produced in abundance.[7]

The differences here noted upon these different media are sufficient to alter entirely the general appearance and to shift the fungus from the Tuberculariaceae-Dematiae to the Tuberculariaceae-Mucedinae.

CONIOTHYRIUM FUCKELII SACC., FROM APPLE

This fungus when growing upon a medium rich in starch becomes black in its peripheral layer. Glucose fails to produce the same

[7] N. C. Agric. Exp. Sta. Bull. 196. June, 1907.

result. The mycelium is hyaline when on pea agar, but tawny on apple agar.[8]

SEPTORIA PETROSELINI VAR. APII, FROM CELERY

This fungus fails to produce naked spores when sown thickly on celery agar though it does so under similar conditions when upon lettuce agar.

COLLETOTRICHUM CARICA S. & H., FROM FIG

This fungus upon the different media used showed striking differences in: number of setae, varying from none to abundant; number of spores, varying from few to many; color, varying from pale to almost black.

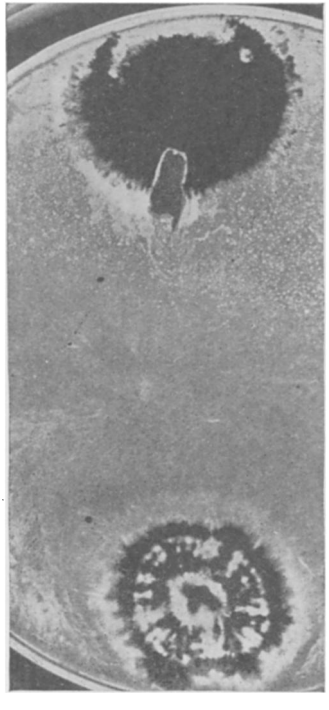

FIG. 11.—*Volutella fructi* S. & H.; two black colonies upon CBA + sodium asparaginate + starch.

On CBA growth was scant; acervuli small, setae absent.

On CBA+ammonium lactate and CBA+sodium asparaginate, growth was about as in CBA, except that numerous black setae were present.

On CBA+ammonium lactate+ starch, the acervuli were larger, more numerous, with numerous large black setae.

On CBA+sodium asparaginate+ ammonium lactate, there were a few setae.

On CBA + sodium asparaginate + glucose, black setae were numerous.

EPICOCCUS SP., FROM APPLE AGAR IN PETRI DISHES

This fungus on pure agar and CBA was colorless. On CBA + starch or CBA+glucose, there was much richer mycelial development, which moreover took on a rich yellow color that in spots turned to

[8] N. C. Agric. Exp. Sta. Bull. 196:51.

pink. Sometimes black spots developed on the first of these media but not upon the second. This fungus shows strikingly the differentiating value of starch and glucose for fungus culture.

Upon apple agar still another character developed, a rich golden color of the abundant, floccose, matted, aerial hyphae. This reaction is fully as striking as the familiar rose color produced by certain species of Fusarium.[9]

With this fungus we have absence of color in agar and CBA, but rich coloring, of varying hues, in the presence of carbohydrates and upon apple agar.

PHYLLOSTICTA SP., FROM APPLE AGAR IN PETRI DISHES

This fungus grew faster on agar than on CBA, formed pycnidia sparsely on agar and not at all on CBA.

With sodium asparaginate added the mycelium became very dense, with considerable aerial development, remained colorless, and produced few pycnidia, and these visible only with the two-thirds objective. The presence of glucose led to exceedingly profuse pycnidial development, while on starch the growth was as with CBA+sodium asparaginate, showing again the ability to utilize glucose but not starch.

ALTERNARIA SP., FROM LAWSON CARNATION

This fungus, the cause of an apparently undescribed carnation disease which will be the subject of a subsequent paper, was isolated during October, 1908. There were striking differences in the color of the colony upon different media, varying from merely hyaline to dense black. The size and color of the spores were also so modified as to give much more than what is usually regarded as a specific difference.

On pure agar, CBA, CBA+ammonium lactate, CBA+sodium asparaginate, and upon CBA+ammonium lactate+sodium asparaginate, the mycelium was colorless and the colony correspondingly colorless; while upon CBA+sodium asparaginate+starch and CBA +sodium asparaginate+glucose, the mycelium was very dark, more profuse, more freely branched, and the colony therefore of an entirely different aspect.

9 BESSEY, ERNST, Ueber die Bedingungen der Farbbildung bei Fusarium. Inaug. Diss. Halle. 1904.

Spore formation proceeded sparingly, though evenly and regularly, upon pure agar, CBA, CBA+ammonium lactate, CBA+sodium asparaginate, CBA+sodium asparaginate+ammonium lactate; but very abundantly upon CBA+sodium asparaginate+starch and upon CBA+sodium asparaginate+glucose. Here the sodium asparaginate seems not to furnish the carbon in sufficiently available form, though starch or glucose do so to nearly equal extent.

The size, color, and septation of the spores were also greatly influenced by the medium. From carnation-agar plates the spores measured 16 to 52 μ long by 6 to 13 μ thick, bearing 0 to 3 longitudinal septa and three to seven transverse septa; while from the live carnation leaf the spores were 26 to 123 μ long by 10 to 20 μ thick, bearing 1 to 9 or often numerous longitudinal septa and 3 to 15 transverse septa. It is seen that the spores are approximately twice as long, twice as thick, of darker color, and with many more septa in each direction upon the natural medium than upon the carnation agar, differences which would ordinarily be regarded as clearly of specific rank.

ALTERNARIA BRASSICAE (BERK.) SACC., FROM COLLARD

This fungus made hyaline mycelium in CBA and CBA+sodium asparaginate; black mycelium in CBA+sodium asparaginate+glucose and in CBA+sodium asparaginate+starch, starch producing by far the most pronounced effect.

Digestion of the starch grains, somewhat in advance of the tips of the oncoming fungous threads, produced a clear zone surrounding each colony in the starch-bearing plates.

ASCOCHYTA CHRYSANTHEMI STEVENS

This fungus was grown in the usual media with no significant effects, except that the fungus did not digest the starch grains afforded in the medium.

A deposit of great thickness around mycelial threads was made in the case of certain media and not in others, as has already been noted.[10]

In some instances culture at a high temperature occasioned this same reponse.

[10] BOT. GAZETTE 44:241. 1907.

SUMMARY OF CHEMICAL RELATIONS NOTED

The most striking response to chemicals is in color, which so far as observed was invariably heightened by the presence of chemicals bearing carbon in available form, the form of available carbon varying for different fungi. Some fungi, possessing ability to digest starch, can utilize this as a source, while to others the carbon of starch is inaccessible. Special unknown chemicals in apple add vivid colors to fungi otherwise hyaline. Some chemicals also promote or inhibit spore formation. Some inhibit or promote growth of setae and some even alter the size, color, and septation of spores. MILBURN,[5] working under KLEBS, has also noted pronounced effects of chemicals upon the color of fungi. The difference in color effects produced by different fungi under the same conditions, and with the same fungus under different conditions, is also noted by BESSEY.[9] No correlation is noted between rapidity of lineal growth and nutritive value of the medium. In many instances most rapid lineal growth occurred in what was surely the poorest medium. Very poor media suffice in many cases also for spore formation, while rich media often result in cessation of spore formation.

Colletotrichum Lindemuthianum, sometimes with setae, often without, has long been of questionable generic position. The same is true of several other species of this genus. *Alternaria Brassicae* and *Macrosporium Brassicae* agree closely except as to presence or absence of catenulate spores.[11] Variation of this kind is probably due to variation in chemical composition of the supporting medium, e. g., change in sugar content as ripening proceeds, acting in such way as to give the fungus the appearance of belonging to one genus when upon the green sugar-tree fruit, to another genus as the starch gives place to sugar as the fruit ripens.

IV. Light relation

The absence of material effect of light upon lineal growth with these species of fungi is shown in Table I.

Ascochyta Chrysanthemi Stevens.—The growth is more floccose in darkness.

Phyllosticta sp.—This fungus forms its pycnidia in beautiful con-

[11] *A. Brassicae* "hyphis brevibus conidiis 60–80×14–18 μ, 6–8 septato-muralibus." *M. Brassicae* "hyphis obsoletis conidiis 50–60×12–14 μ, 5–11 septatis."

centric rings when in open room, i.e., alternate light and darkness, but in continuous darkness they were irregularly scattered. Culture no. 35 made concentric rings when in the light, and failed to do so

TABLE I

Relation of light to growth

Figures express growth in millimeters. The cultures marked "alternate" were kept several days in light and several days in dark; *L* light; *D* dark. Inoculated Dec. 8, 1908.

	LIGHT CONDITION	DECEMBER										
		9	10	11	12	13	14	15	16	17	18	19
Macrosporium Brassicae	In light.....	germ	1		6	9	13	16	17	23	26	28
	Alternate light & dark	*L*	*L*	*L*		*L*	*D*	*D*	*D*	*L*	*L*	*L*
		germ	1		6	10	12	15	17	23	26	28
	In dark.....	germ	1		6	9	11	15	17	21	29	29
Phyllosticta sp.	In light.....	0	gr.		4	7	10	13	14	16	20	23
	Alternate light & dark	*L*	*L*	*L*	*L*	*L*	*D*	*D*	*D*	*L*	*L*	
		0	gr.		4	7	10	13	13	16	18	20
	In dark.....	0	gr.		4	6	7	10	13	16	19	20
Ascochyta Chrysanthemi	In light.....		4		12	15	17	25	26	33	39	41
	Alternate light & dark	*L*	*L*	*L*	*D*	*D*	*D*	*D*	*D*	*L*	*L*	*L*
		2	3		12	16	20	25	30	37	39	45
	In dark.....				12	14	18	22	25	31	37	37

when moved to darkness. Cultures kept in the open room lay down rudiments of pycnidia mainly during the night, and it is probable that light exerts enough inhibiting influence on pycnidial development to give a growth predominance during the day and a fructifying predominance during the night (HEDGECOCK, *l. c.*[4]).

Alternaria Brassicae (Berk.) Sacc.—With this fungus the end of each day's growth, evening, marks the edge of a zone. The zone thus marked is intensified during the succeeding twenty-four hours by color changes. While zones are formed to some extent in continued darkness, they are more pronounced in the room condition.

SUMMARY OF LIGHT RELATION

Light exerts little or no effect upon lineal growth with these fungi. It appears to exert an inhibiting influence on pycnidial development and in some instances is the cause of zonation in colonies.

V. Unknown factors

Ascochyta Chrysanthemi Stevens

This fungus frequently exhibited differences in character along different radii of the same colony, the conditions of medium, thickness of sowing, humidity, etc., being apparently identical.

Fig. 12 shows such a colony. Along the radius *aa*, at *b*, the colony bore pycnidia abundantly, and the mycelial progeny of this strain extending to the periphery of the colony was rich in pycnidia, while

FIG. 12.—*Ascochyta Chrysanthemi* Stevens, showing abundant pycnidia on radius *aa*, at the point *b*, and paucity of pycnidia elsewhere.

most other radii of the colony were sterile or nearly so. Transfers were made from the point *c* (sterile) and *d* (pycnidial) to fresh plates. The sterile mycelium produced a colony which was sterile through its early days. As it aged it formed a few large pale pycnidia. The fertile strain produced a fertile colony with very numerous, though

small, pycnidia. Transfers made again from these two strains resulted in a complete reversal of character, the fertile becoming sterile and the sterile becoming fertile. No explanation of this suggests itself.

When this fungus was plated from a suspension of spores, two types of colony developed, corresponding to the two strains mentioned

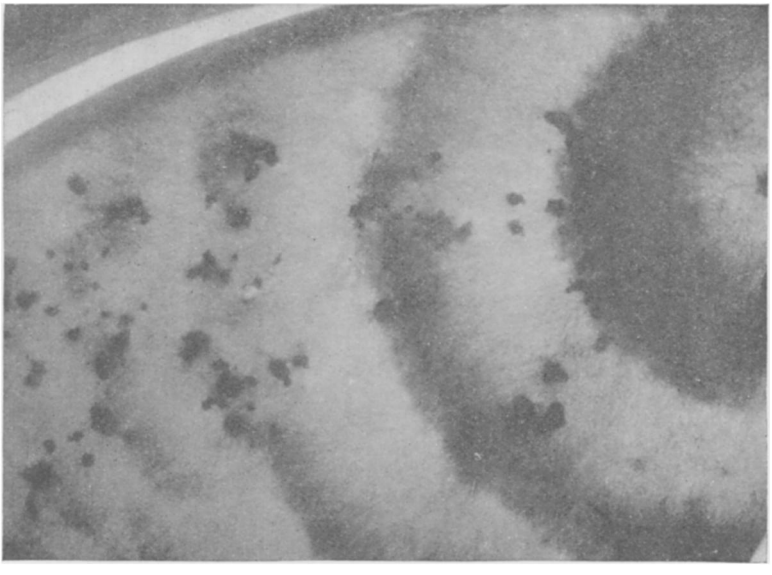

FIG. 13.—*Ascochyta Chrysanthemi* Stevens; portion of colony showing few pycnidia; cf. *fig. 14*.

above. The first "type of few pycnidia" developed a copious aerial mycelium of loose floccose nature, extended regularly in all directions, and was long devoid of pycnidia. When the pycnidia did form, they were few, large, and superficial (*fig. 13*). The second "type of many pycnidia" had little or no aerial mycelium, all the mycelium being either immersed or of strict growth; was roughly circular in colony, not regularly so as in first type; and small, irregular, mostly immersed pycnidia were formed in myriads throughout the colony (*fig. 14*). These two types of colony appeared on the same plates which were inoculated with spores from the same pycnidium. They therefore developed in the same nutrient condition, humidity, temperature, etc. Depth of planting was not the cause of these differences,

since flooding the plate with an extra tube of agar after the agar first plated had set, did not change the proportion of the two types. Nor did sowing in such way that the spores were at the bottom rather than at the top of the agar change results. There was a marked tendency of colonies of both types of the fungus to become more productive of large pycnidia where two different colonies approach each other, sug-

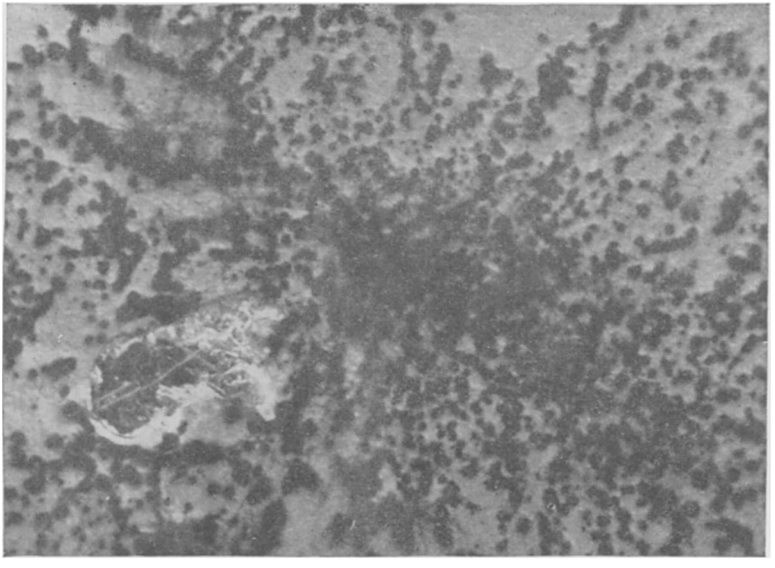

FIG. 14.—*Ascochyta Chrysanthemi* Stevens; portion of colony showing many pycnidia; cf. *fig. 13*.

gesting that there might be needed a cooperation of two diverse strains in order to form a pycnidium; that the strains of few pycnidia lacked the requisite individuals, and that the strains of many pycnidia had more than one individual to the colony. To test this, colonies were traced from the earliest development, resulting in clear evidence that in some instances a colony developed from a single spore was one with few pycnidia; in other instances a single spore produced a colony of many pycnidia.

CONIOTHYRIUM FUCKELII SACC., FROM APPLE

In one instance this fungus, which rarely fruited, made pycnidia in almost perfect circles near the margins of each colony on the plate (*fig. 15*).

These variations are inexplicable and remind one of the mysterious change from the ascigerous to the non-ascigerous condition so frequently met in life-history work with the imperfect fungi.

Variability in spore measurements

Since the beginning of mycology it has been customary to give spore measurements in specific description, probably originally with

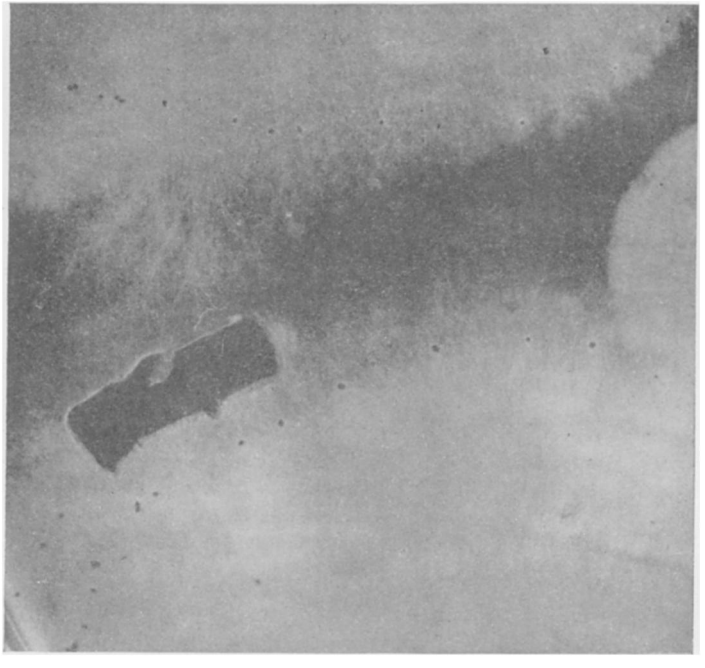

FIG. 15.—*Coniothyrium Fuckelii* Sacc.; portions of two colonies showing circles of pycnidia near margins.

the idea of giving some information as to the approximate size of the plant concerned rather than to give exact descriptive limitations. With the advance of time, great importance has come to be attached to spore measurements, greater perhaps than is warranted, and many species are now founded upon divergence in this one character—and often upon slight divergence.

To ascertain the variability in spore measurement under constant conditions and its variability as occasioned by changes in environment, studies with several species of fungi were undertaken.

The measurements were all made in water in which the spores had stood long enough to become fully turgid, taking only such spores as were completely ripe, as was shown by the fact that they were extruded from the pycnidium, ascus, or sporodochium naturally, without assistance. An eyepiece micrometer was used and the units here employed are usually one division of the eyepiece scale (equal to 3.7 μ), which constituted in most cases as small a unit as could be used to advantage. Spore measurements involving half the division were recorded as with the next lower integer unless otherwise designated. To avoid any possibility of unconscious selection, the spore lying closest to contact with the end of the micrometer scale at the completion of a measurement was taken for the next measurement. In the polygons each small square (one 256th of a square inch) represents one spore.

We wish to acknowledge our indebtedness to Dr. G. H. SHULL, who has kindly read this portion of the manuscript, for calculating the constants, and to Mr. B. B. HIGGINS, by whom most of the measurements were made and upon whose very accurate and painstaking work the value of the measurements depends.

ASCOCHYTA CHRYSANTHEMI STEVENS

A. *Spores from the large pycnidium type* (see p. 18)

Pycnidium no. 1. A large pycnidium produced in a colony which had very few pycnidia.

$M = 4.9645 \pm 0.0393$
$\sigma = 0.9787 \pm 0.0278$
C. V. $= 19.714 \pm 0.581$
$n = 284$

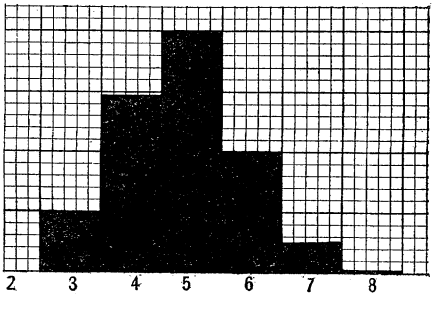

FIG. 16. — *Ascochyta Chrysanthemi* Stevens. Polygon of spores from pycnidium no. 1, large type. 3 should cover 20 squares instead of 25.

Pycnidium no. 2. Large type.

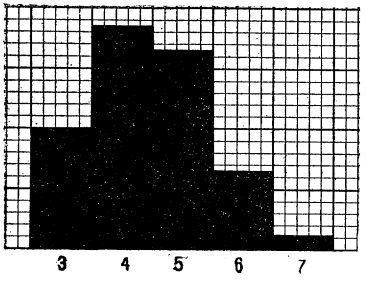

FIG. 17.—*Ascochyta Chrysanthemi* Stevens. Polygon of spores from pycnidium no. 2, large type.

$M = 4.4318 \pm 0.0398$
$\sigma = 0.9589 \pm 0.0281$
C. V. $= 21.638 \pm 0.650$
$n = 254$

Pycnidium no. 3. From a plate bearing one large colony. The whole colony was characteristically one of few pycnidia, which were of large type and light color. The spores were obtained without any possibility of the pycnidium being torn; that is, they were normally ripe spores.

$M = 3.3848 \pm 0.0245$
$\sigma = 0.6714 \pm 0.0173$
C. V. $= 19.836 \pm 0.531$
$n = 343$

It is seen that these three separate pycnidia of the same type gave modes of 4.9645 ± 0.0393, 4.4318 ± 0.0398, and 3.3848 ± 0.0245; or, expressed in terms of the systematist, that in the three pycnidia the spores measured 11.1–29.6 μ, mostly 18.5 μ; 11.1–25.9 μ, mostly 14.-8 μ; 7.4–22.2 μ, mostly 11.1 μ; showing that measurements from one pycnidium alone are not sufficient for reliable characterization.

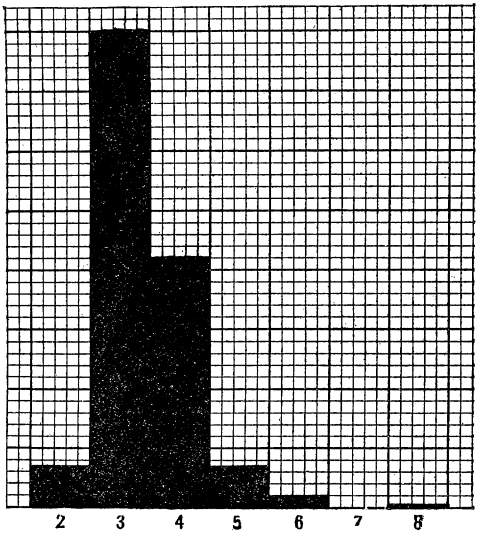

FIG. 18.—*Ascochyta Chrysanthemi* Stevens. Polygon of spores from pycnidium no. 3, large type.

B. *Spores from small pycnidium type* (see *fig. 14*)

Pycnidium no. 4. Small type.

M= 3.6011±0.0363
σ= 0.7183±0.0256
C. V.=19.947 ±0.740
n=178

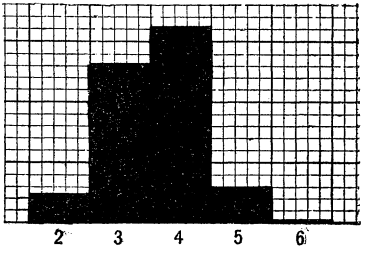

FIG. 19.—*Ascochyta Chrysanthemi* Stevens. Polygon of spores from pycnidium no. 4, small type.

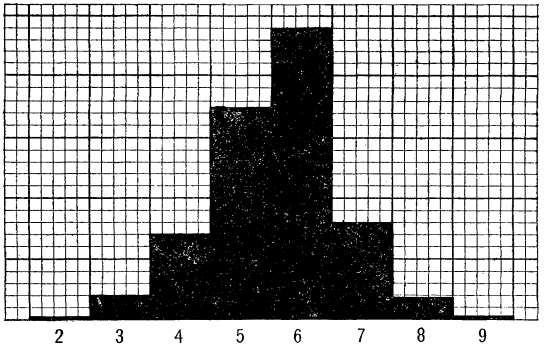

FIG. 20.—*Ascochyta Chrysanthemi* Stevens. Polygon of spores from pycnidium no. 5, small type.

Pycnidium no. 5. Spores taken from small pycnidia from colony shown in *fig. 14*.

M= 5.5850±0.0414
σ= 1.0737±0.0293
C. V.=19.225 ±0.543
n=306

Pycnidium no. 6. A very small pycnidial type.

M= 5.3629±0.0544
σ= 1.2711±0.0385
C. V.=23.702 ±0.756
n=248

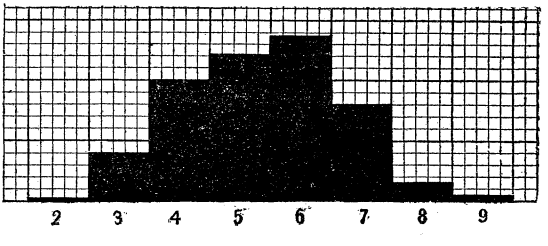

FIG. 21.—*Ascochyta Chrysanthemi* Stevens. Polygon of spores from pycnidium no. 6.

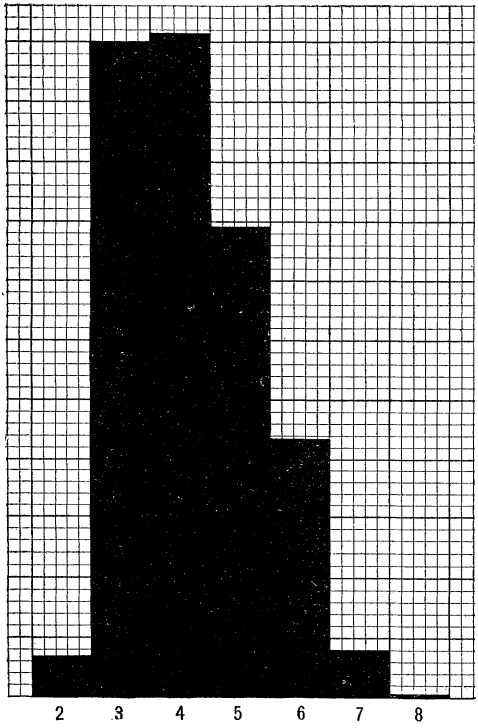

FIG. 22.—*Ascochyta Chrysanthemi* Stevens. Polygon of spores of large pycnidia.

Collecting the data from the large pycnidium type in one polygon, and similarly with the small pycnidium type we have:

$$M = 4.1935 \pm 0.0247$$
$$\sigma = 1.0902 \pm 0.0174$$
$$C.\ V. = 25.998 \pm 0.443$$
$$n = 889$$

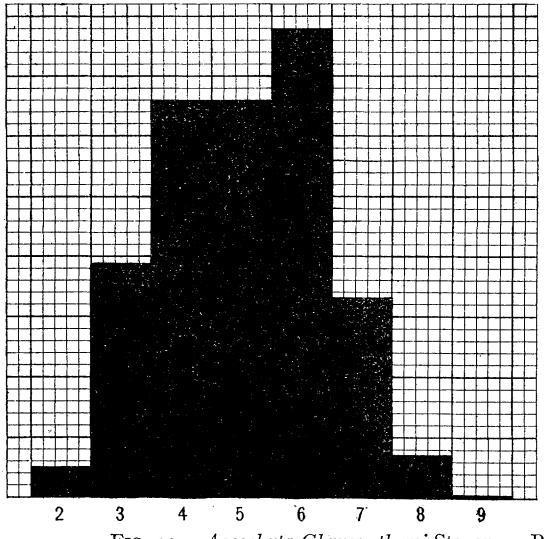

FIG. 23.—*Ascochyta Chrysanthemi* Stevens. Polygon of spores of small pycnidia.

$$M = 5.0379 \pm 0.0335$$
$$\sigma = 1.3492 \pm 0.0237$$
$$C.\ V. = 26.781 \pm 0.503$$
$$n = 738$$

It is seen that there is a tendency throughout for the smaller pycnidia to produce larger spores than are produced by the large pycnidia.

C. *Measurements of spores from different media*

Pure agar. The pycnidia on this plate were very scant, although they were normal in appearance and size.

$M = 2.6241 \pm 0.0313$
$\sigma = 0.5354 \pm 0.0221$
C. V. $= 20.402 \pm 0.878$
$n = 135$

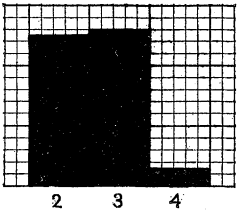

FIG. 24.—*Ascochyta Chrysanthemi* Stevens. Polygon of spores from pure agar.

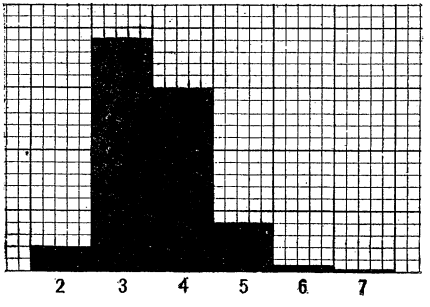

FIG. 25.—*Ascochyta Chrysanthemi* Stevens. Polygon of spores from CBA + 0.25 per cent. sodium asparaginate.

CBA + 0.25 per cent. sodium asparaginate.

$M = 3.5637 \pm 0.0358$
$\sigma = 0.7579 \pm 0.0253$
C. V. $= 21.267 \pm 0.725$
$n = 204$

CBA + sodium asparaginate + starch.

$M = 5.4267 \pm 0.0355$
$\sigma = 0.7896 \pm 0.0251$
C. V. $= 14.551 \pm 0.459$
$n = 225$

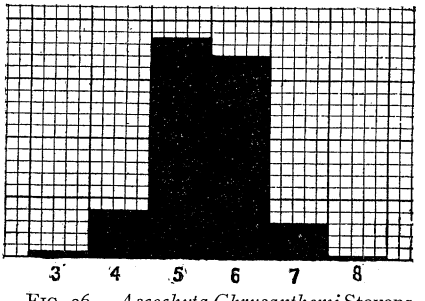

FIG. 26.—*Ascochyta Chrysanthemi* Stevens. Polygon of spores from CBA + sodium asparaginate + starch.

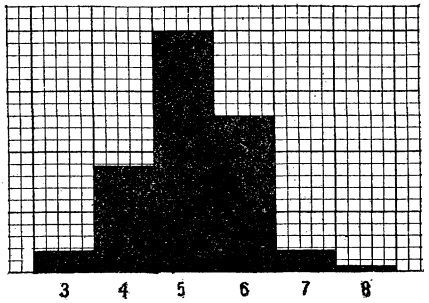

FIG. 27.—*Ascochyta Chrysanthemi* Stevens. Polygon of spores from CBA+sodium asparaginate+glucose.

CBA+sodium asparaginate+1 per cent. glucose. This was a remarkable colony with spores distinctly smoky or olivaceous.

$M = 5.1422 \pm 0.0408$
$\sigma = 0.9214 \pm 0.0289$
C. V. $= 17.919 \pm 0.579$
$n = 232$

Plated thickly in 4 per cent. pea agar.

$M = 4.3246 \pm 0.0392$
$\sigma = 1.0138 \pm 0.0277$
C. V. $= 23.442 \pm 0.674$
$n = 350$

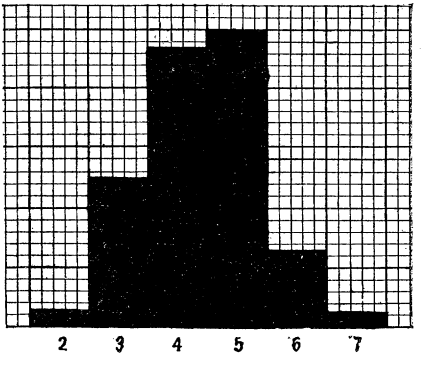

FIG. 28.—*Ascochyta Chrysanthemi* Stevens. Polygon of spores from 4 per cent. pea agar.

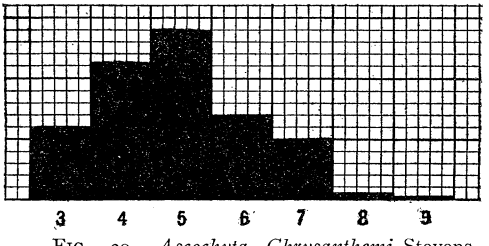

FIG. 29.—*Ascochyta Chrysanthemi* Stevens. Polygon of spores from cow pea agar.

Cow pea agar.
$M = 4.8657 \pm 0.0545$
$\sigma = 1.1885 \pm 0.0386$
C. V. $= 24.427 \pm 0.839$
$n = 214$

It is seen that on these different media the mode varies materially, being low on pure agar, higher on CBA+sodium asparaginate, and still higher when glucose or starch is added. The mode is high also in natural media, such as pea agar and cow pea agar.

In the terms of the systematist, spores from pure agar measured 7.4–14.8 μ, mostly 11.1 μ; those from CBA+sodium asparaginate 7.4–25.9 μ, mostly 12.9 μ.

SEPTORIA LYCOPERSICI SPEG. OF TOMATO

Grown on apple agar.

$M = 21.507 \pm 0.190$
$\sigma = 4.686 \pm 0.135$
C. V. $= 21.787 \pm 0.655$
$n = 278$

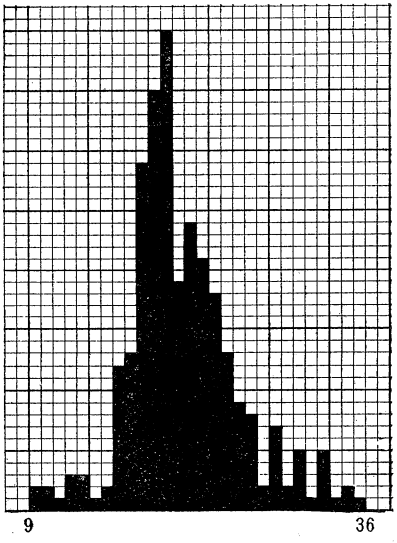

FIG. 30.—*Septoria Lycopersici* Speg. Polygon of spores on apple apar.

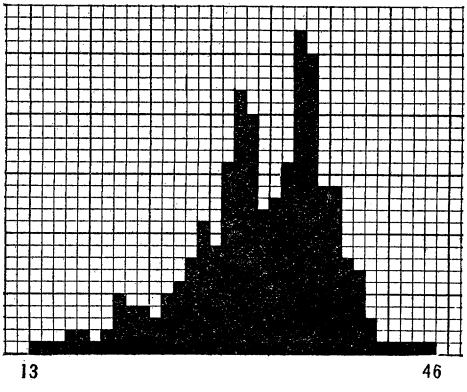

FIG. 31.—*Septoria Lycopersici* Speg. Polgyon of spores on pure agar.

Grown on pure agar.

$M = 31.675 \pm 0.242$
$\sigma = 5.879 \pm 0.171$
C. V. $= 18.560 \pm 0.559$
$n = 279$

Although the number of spores measured in the two last instances is not large, the fact of a tendency to larger spores on the poorer medium, apple agar, is evident. The spores measured 33.6–133.2 μ, mostly 81.4 μ; those on pure agar 48.1–181.3 μ, mostly 133.2 μ.

Ascospores of Sclerotinia Libertiana Fuckel

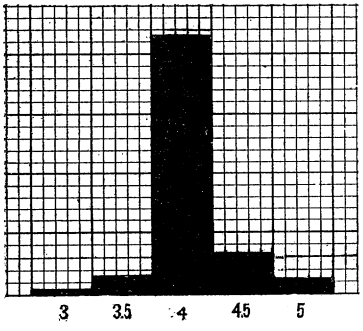

Fig. 32.—*Sclerotinia Libertiana* Fckl. Polygon of ascospores from middle-aged disk.

Spores were discharged spontaneously from the disk upon the cover glass, the disk being of middle age.

$M = 4.0880 \pm 0.0166$
$\sigma = 0.2930 \pm 0.0117$
C. V. $= 7.168 \pm 0.290$
$n = 142$

Spores were secured as in the last instance, but from very young disks.

$M = 4.0393 \pm 0.0214$
$\sigma = 0.3743 \pm 0.0151$
C. V. $= 9.267 \pm 0.380$
$n = 165$

No material difference in the size of the spores here appeared with the change in age of the disks.

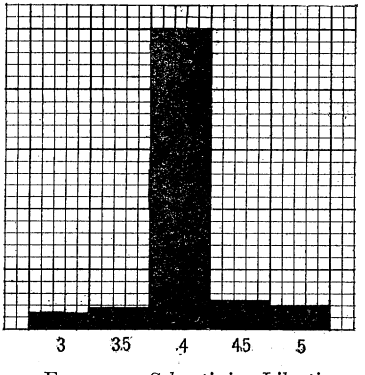

Fig. 33.—*Sclerotinia Libertiana* Fckl. Polygon of spores from young disk.

DIPLODIA MACROSPORA EARLE

Spores of this species, isolated from corn, were grown upon pea agar.

$M = 24.362 \pm 0.176$
$\sigma = 3.179 \pm 0.124$
C. V. $= 13.050 \pm 0.519$
$n = 149$

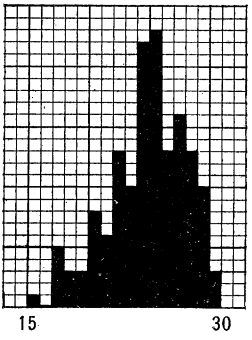

FIG. 34. — *Diplodia macrospora* Earle. Polygon of spores isolated from corn and grown upon pea agar; measurements showing length.

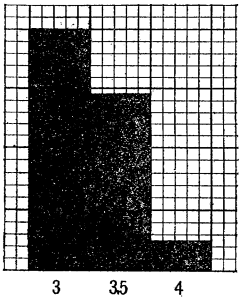

FIG. 35.—*Diplodia macrospora* Earle. Polygon of spores isolated from corn and grown upon pea agar; measurements showing thickness.

$M = 3.2595 \pm 0.136$
$\sigma = 0.2727 \pm 0.0096$
C. V. $= 8.367 \pm 0.297$
$n = 183$

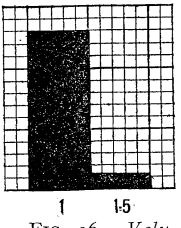

FIG. 36.—*Volutella fructi* S. & H. Polygon of spores showing width.

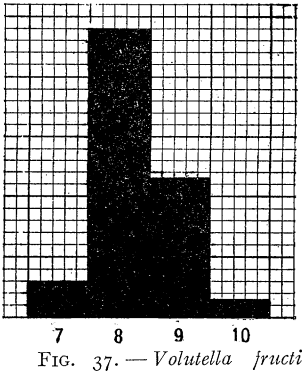

FIG. 37. — *Volutella fructi* S. & H. Polygon of spores showing length.

VOLUTELLA FRUCTI S. & H.

$M = 8.27 \pm 0.0276$
$\sigma = 0.5778 \pm 0.0195$
C. V. $= 6.986 \pm 0.237$
$n = 200$

The last five polygons are without particular significance and serve only to show the variation encountered in these forms.

General considerations

The bearing of these facts upon mycological taxonomy is apparent. If a fungus can be easily changed as regards its essential descriptive characters by a change in substratum, density of infection, or other environmental factor, these characters are worthless for descriptive purposes, unless the conditions under which they develop be accurately known.

There are two fundamental benefits from description: (1) to enable recognition of a particular form; (2) to aid in classification. The first of these is a necessary preliminary to the second, and it is with mere recognition that we have in many instances yet to deal in mycology, particularly among the group *Fungi imperfecti*, with its enormous genera, such as Septoria, Phyllosticta, and Cercospora, with their thousands of so-called species. While life-history work and infection experiments will do much, accurate recognition of the form in hand is a necessary preliminary even to this.

To reach any satisfactory basis, many fungi must be studied in culture, under suitable standard conditions, much after the fashion that bacteria are now studied.

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